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CHAPTER 1

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Keywords: Adjuvant cancer therapy, bisphosphonates, clodronate, fibroblasts, immune cells, immunotherapy, liposomes, macrophage depletion, macrophages, myeloid derived suppressor cells, neutrophils, repolarization, reprogramming, stromal cells, stromal interactions, therapeutic targets, tumor associated macrophages, tumor associated neutrophils, tumor microenvironment.

INTRODUCTION

Cancer progression mostly depends on the ability of malignant cells to exploit physiological processes of the host. Solid tumors can only develop with a steady

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supply of nutrients and oxygen, provided by blood and by support of cells, factors and conditions provided by the microenvironment [1-26]. Cells of the microenvironment become activated by communication with the tumor cells, consequently creating numerous conditions that promote cancer growth and ultimately lead to metastatic dissemination [5, 27-38].

First evidences about the effect of the host microenvironment on tumor growth were provided in the 1970s [39, 40] postulating that expansion from a single mutated cell to a solid tumor can only occur when the stromal environment is altered in a way to allow unrestrained tumor growth. Despite of continuous efforts, for many years cancer research largely focused on cancer-cell driven carcinogenesis and on understanding the mutations causing neoplastic cell transformations. This cancer cell centric view of tumor progression largely ignored the fact that complex interactions between cancer cells and stromal components tightly regulate and orchestrate tumor growth and metastatic dissemination. For this and other reasons, even after decades of implementing treatments that selectively target the tumor cell, survival of metastatic cancer patients is still disappointingly short. Therefore, novel strategies are urgently needed to complement the classical treatment modalities with new therapeutic approaches. In this regard, interactions between cancer cells and their host environment offer novel opportunities for therapies based on the improved understanding of the nature of these interactions and the mechanisms that govern them. Treatment modalities that target both cancer cells and components of the tumor microenvironment (TME) are likely to be more effective than those classically directed against cancer cells. A potential advantage in targeting the non-malignant cells of the TME is that these cells tend to be more genetically stable and are therefore less expected to develop resistance to therapies.

To provide new therapeutic strategies targeted at the immune components of the TME, it is critical to understand how these cells are altered during tumor progression and how they reciprocally influence tumor initiation, progression and metastasis. Here, we review the current understanding of the interactions of tumor cells with the microenvironment with a particular focus on tumor associated macrophages (TAM), tumor associated neutrophils (TAN) and myeloid derived suppressor cells (MDSC) (Fig. **1**). Current therapeutic approaches aiming at the

TME, in particular cell-based therapies and therapies with bisphosphonates (BP), a class of drugs that show to have potential immunomodulatory properties on immune cells in cancer, are reviewed and discussed. Their pharmacological properties and anti-tumor activities are summarized with a special emphasis on the properties of clodronate encapsulated in liposomes, a drug formulation that has the ability to deplete tumor-associated macrophages. Together, all these properties point toward the significance of re-programming myeloid cell phenotypes to affect tumor growth and accordingly, suggest this concept as a promising strategy to complement the established anticancer treatment modalities.

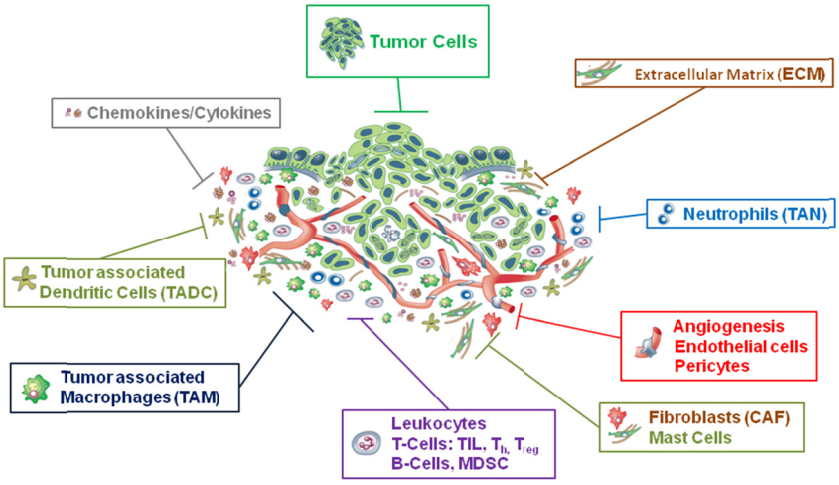

Figure 1: The tumor microenvironment (TME) is composed of numerous different cell types that infiltrate a growing tumor. These cell types include vascular or lymphatic endothelial cells, endothelial cell supporting pericytes, fibroblasts, mast cells, and the cells of the innate and adaptive immune system, namely macrophages, dendritic cells, neutrophils, leukocytes (T cells, B cells) and myeloid derived suppressor cells (MDSC). In addition, the non-cellular components of the TME include components of the extracellular matrix (ECM) and soluble factors as chemokines and cytokines. The therapeutic strategies of targeting components of the TME include the tumor cells themselves by combining novel adjuvant therapy approaches targeted to cellular or molecular components of the TME with the current chemotherapy and radiotherapy. Novel and experimental therapies that aim at components of the TME include inhibitors of angiogenesis (*e.g.* anti-VEGF or VEGF-receptor antibodies), inhibitors of fibroblast functions, drugs aimed at macrophages and neutrophils (depletion, re-polarization), immune stimulating therapies (antibodies, cellular therapies, vaccines), inhibitors of EMC components (*e.g.* MMP inhibitors) and inhibitors of chronic inflammation.

Characteristics and Components of the Tumor Microenvironment

Angiogenesis, Hypoxia and Oxygen Regulation

Angiogenesis is a key process for tumor development. Small colonies of malignant cells of 1-2 mm^3 size, the so-called "carcinoma-*in-situ*", alter their phenotype to induce continuous proliferation of endothelial cells and development of new blood and lymph vessels. This "angiogenic switch" triggers the expansion of the tumor cells by growth of new vessels that provide nutrients, oxygen and removal of waste products, as well as an escape route for metastasizing tumor cells [22, 24, 41-47].

Although various studies demonstrated that tumor cells produce pro-angiogenic factors, angiogenesis is also stimulated by activated myeloid cells recruited into the neoplastic tissue. Production of vascular endothelial growth factor (VEGF) is an important mechanism by which tumor infiltrating myeloid cells trigger and enhance angiogenesis and foster tumor development [48, 49]. TAMs are a major source of VEGF as they accumulate in poorly vascularized hypoxic areas and respond to hypoxia by releasing VEGF and other angiogenic factors (see below and Fig. **2**). Hypoxic conditions in tumors stimulate the expression of pro-angiogenic molecules by activating hypoxia-inducible factors (HIFs) in macrophages [19, 50-58]. Activated macrophages also release nitric oxide (NO), a molecule that provokes increased vascular flow [46, 59-64]. Myeloid derived suppressor cells (MDSC, see below) represent another cell population involved in tumor angiogenesis. Tumor cell educated MDSCs express elevated levels of the matrix degrading metalloproteinase MMP-9 that triggers VEGF release from the extracellular matrix (ECM) which induces proliferation of endothelial cells [65-68]. Despite of their low abundance, tumor associated neutrophils (TANs, see below) have also been reported to support tumor growth by producing pro-angiogenic factors such as VEGF, IL-8 and proteases including MMPs and elastase [65, 69-73]. In this context, it was found that Stat3 activation in tumor-associated myeloid cells is critical for tumor angiogenesis [74]. Last but not least, pericytes, responsible for the stabilization of endothelial cells of the vessel wall, play a crucial role in hem- and lymphangiogenesis where they closely interact with endothelial cells and vascular smooth muscle cells [75-78]. Although the importance of myeloid cells in promoting tumor angiogenesis has been

investigated carefully, the underlying molecular mechanisms as well as the individual contributions of the different cell types remain to be fully explored.

The Extracellular Matrix (ECM) and Regulation of Invasion and Metastasis

The ECM serves as a scaffold for the cellular components of normal tissues as well as of tumors and it also strongly influences cell growth, differentiation, adhesion, motility, invasion and viability. The ECM consists of proteins that possess multiple functions and that provide vital signals for tumor progression and metastatic spread [79-85]. The matrix metalloproteinases (MMPs) with their proteolytic activity are key modulators of the TME and the most prominent family of proteases associated with tumorigenesis. They play an important role in ECM turnover and remodeling and in tumor cell migration. MMPs also control signaling pathways that regulate cell growth, inflammation and angiogenesis [86-88]. The transmission of signals between the ECM and neighboring cells occurs mainly through the integrins. These proteins have the capability to transduce mechanical cues created by the ECM or the cell cytoskeleton into chemical signals that regulate many cellular processes such as proliferation, survival, migration, and invasion [80, 82, 84, 89, 90].

An important step in tumor progression is the acquisition of invasive properties by tumor cells. Epithelial-mesenchymal transition (EMT) is a well characterized mechanism, through which epithelial cells trans-differentiate and acquire an invasive, fibroblast-like phenotype [32, 91-95]. Although it is well established that the TME contains cytokines, growth factors and enzymes that induce EMT, the cellular sources of these factors remain to be fully identified. TAMs, cancer-associated fibroblasts, CAFs, mesenchymal stem cells (MSCs) and lymphocytes have all been shown to contribute to an EMT promoting tumor microenvironment [95, 96]. Pro-inflammatory macrophages have likewise been shown to induce EMT at the invasive front, but also in the core of tumors, mainly through stabilization of Snail and Smad3, key mediators of EMT [97-99].

The ability of a growing tumor to invade tissue and to metastasize to distant organs was thought to be strictly cancer cell intrinsic. However, it is now established that tumor infiltrating and resident myeloid cells significantly contribute to tumor progression. Myeloid cell subsets as macrophages, MDSCs,

neutrophils and mast cells as well as soluble factors play an important role in ECM remodeling, invasion and metastasis which will be discussed in the forthcoming paragraphs.

Chemokines and Cytokines

The TME is rich in chemokines and cytokines which are vital factors for the regulation of tumor growth, invasion and metastasis. Most of resident and infiltrating cellular components of the TME contribute to a dynamic chemokine/cytokine network which is spatially and temporally fluctuating, depending on the local conditions of the TME. Beyond activating tumor vascularization, infiltrating myeloid cells also promote tumor growth by creating a microenvironment that is rich in growth factors and pro-inflammatory cytokines that stimulate proliferation and survival of neoplastic cells [26, 36, 70, 100-110]. Myeloid cell-derived cytokines and growth factors secreted by TAMs and MDSCs such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF) all contribute to tumor growth [111]. Besides directly promoting tumor cell proliferation, tumor-educated myeloid cells can also indirectly facilitate tumor growth through suppression of anti-tumor immune responses by secretion of immunosuppressive cytokines, generation of reactive oxygen species (ROSs) and increased activity of arginase and nitric oxide (NO). Another important immunosuppressive mediator, TGF-β converts naive $CD4^+$ T cells to adaptive regulatory T cells [112, 113].

Fibroblasts and Mast Cells

Cancer associated fibroblasts (CAF) are a heterogeneous cell population. The main progenitors of activated fibroblasts in the TME are originating from resident fibroblasts. CAFs can also stem from pericytes, smooth muscle cells and from bone marrow derived mesenchymal cells [114, 115]. CAFs contribute to a pro-tumorigenic environment through interaction with other cells in the TME. They are regulators of tumorigenesis and they differ from tumor cells by being more genetically stable. CAFs have properties to enhance tumor angiogenesis by secretion of stromal cell-derived factor 1 (SDF-1), also known as CXCL12, which plays a central role in the promotion of tumor growth and angiogenesis [116]. Besides that they produce many growth

factors (HGF, VEGF, TGF-β), cytokines (IL-8, CXCL14, CCL7, IL-6, IL-1α), proteases (MMPs, uPA) and other enzymes [117]. The clinical relevance of CAFs tumor growth promoting role has also been recognized by exploiting CAF expressed factors as prognostic markers [114, 116, 118-122].

Mast cells (MC) are derived from the bone marrow and are also a heterogenous cell population with many functions. Apart from their role in innate and adaptive immunity they influence tumor cell proliferation and invasion and modulate the immune responses to tumor cells [123]. The number of tumor infiltrating mast cells correlates with increased intratumoral microvessel density, enhanced tumor growth and invasion, and poor clinical outcome. MCs are predominantly located at the boundary between healthy tissues and the TME and are often found in close association with blood vessels. They support angiogenesis by expression of pro-angiogenic factors and by inhibition of ECM remodeling the MCs support tumor spread and metastasis. Tumor-associated mast cells are also regarded as potential therapeutic targets [124-128] and prognostic factor [129-131].

Leukocytes

Leukocyte infiltration into malignant tissue was first described by the pathologist Rudolf Virchow in 1863 [132]. Solid tumors contain various types and numbers of leukocytes that can represent up to 50% of the tumor mass. The major components of the leukocytic infiltrates in the TME are myeloid cells and B and T lymphocytes [38, 133] as well as regulatory T cells [134-138]. Specifically, myeloid cells are the major component of the leukocytic infiltrates found in tumors. Immune cell infiltration into tumors and the impact the immune cells have on cancer has been named cancer immunoediting or cancer immunosurveillance. This concept that describes the role the immune system plays in cancer development was considered and discussed throughout the last decades. The central principle is that the immune system can prevent tumor development but that it is also able to select tumor variants with reduced immunogenicity, and creating an inflammatory environment that provides tumors with mechanisms to escape immune detection and elimination [139-146]. Initially, the presence of leukocytes in malignant neoplasms was thought to represent the host's immune response to a growing tumor [147]. Yet, solid tumors are mostly recognized as "self" and they do not evoke efficient immune responses

capable of eradicating tumors [148, 149]. In contrast, it was found that these cells are actively recruited to neoplastic tissues by tumor cells and that high numbers of several types of leukocytes are associated with tumor progression [38, 150-152]. Nevertheless in some cancers, the presence of leukocytes is associated with a favorable prognosis [153]. For example, enhanced infiltration of natural killer cells and cytotoxic T cells into tumors has been reported to correlate with a good prognosis in human ovarian, colorectal and gastric cancers [154, 155]. Similarly, cytotoxic activation of lymphocytes, particularly $CD8^+$ T cells in response to tumor growth result in regression [156]. In contrast, as described in more details below, tumor-activated myeloid leukocytes (TAMs, DCs, MDSCs) are known to restrain the protective function of these immune cells with anti-tumor activity and to promote tumor growth.

Macrophages

Macrophages belong to the mononuclear phagocyte system (MPS) which are cells involved in host defense functions, immune reactions, disposal of dead cells and cellular components and synthesis of biologically active compounds such as complement components and prostaglandins [157-159]. The MPS includes precursor cells in the bone marrow, blood monocytes, alveolar, peritoneal and splenic macrophages and Kupffer cells in the liver. Macrophages are extremely versatile cells that can adapt a particular phenotype depending on environmental stimuli. As most of the other cell types that populate the TME, they produce an assorted array of chemokines, cytokines, proteases, angiogenic and other growth factors. As unique property they possess the ability to phagozytose particular matter as dead cells, bacteria, viruses as well as artificial particles like liposomes, nanoparticles and other pharmaceutical drug carriers [157, 160-173]. Macrophages play a very important role in tumor development as they are a major component of the myeloid infiltrate in a tumor microenvironment.

Hence, of all cells of the myeloid lineage, they are among the most studied for their contribution to tumor development. Monocytes circulating in the blood are recruited to tumors by tumor-derived chemotactic factors such as the colony stimulating factors M-CSF and GM-CSF (macrophage and granulocyte-macrophage colony stimulating factor), CCL2 (chemokine C-C motif ligand 2,

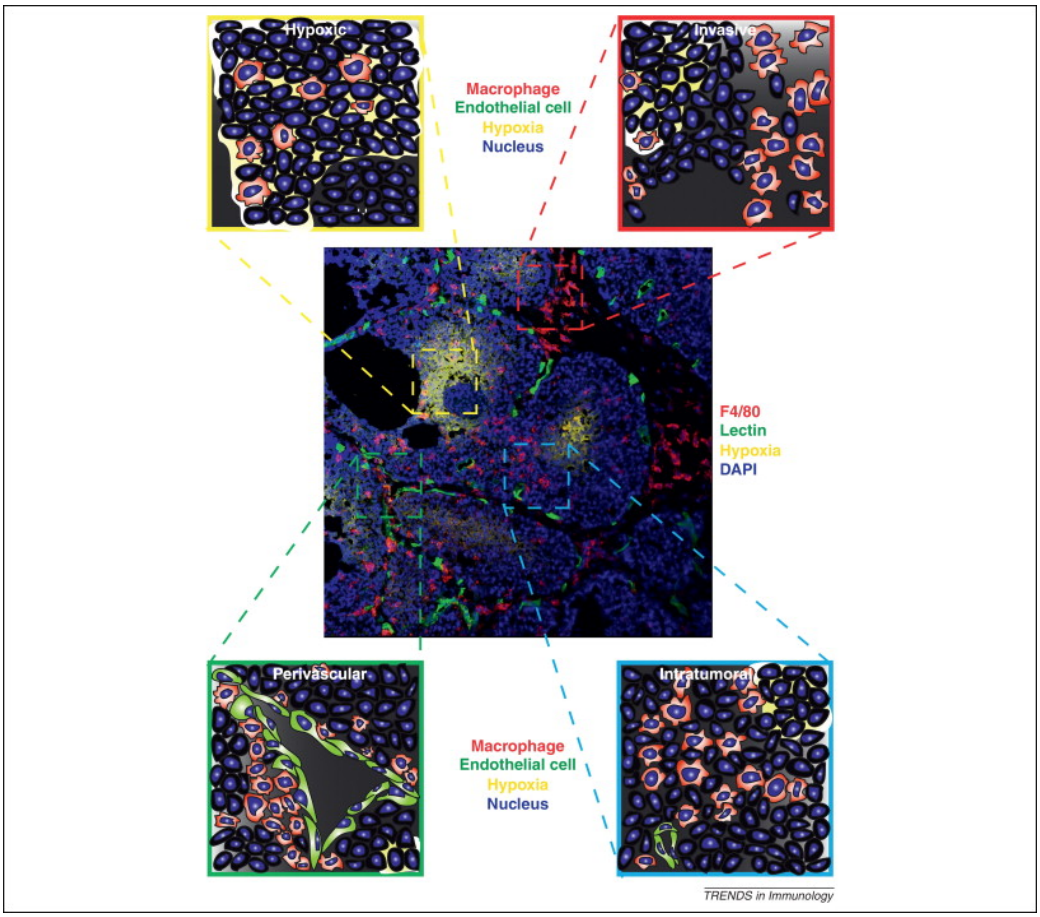

Figure 2: TAM can localize within unique tumor microenvironments. The immunofluorescent confocal micrograph in the center shows red stained F4/80^{+} macrophages within a late-stage tumor of mammary carcinogenesis in the MMTV-PyMT mouse model. Areas of hypoxia are shown in yellow, functional vasculature is stained in green and all cell nuclei are stained in blue with DAPI. Insets display enlarged graphical representations of TAMs within a hypoxic region, at an invasive front, in a normoxic area within the tumor, and associated with the vasculature. Adapted from TRENDS in Immunology with permission [192].

MCP-1) and VEGF. Upon migrating into the tumor the monocytes differentiate into tissue-resident macrophages termed tumor-associated macrophages (TAMs) [38, 46, 59-63, 98, 174-191]. The term TAM defines localization of macrophages at the tumor-stroma interface and in the tumor core. As depicted in Fig. **2**, TAMs localize at different sites in a tumor where they assume different functions that are driven by signals they obtain from the particular microenvironment in which they are located [192]. In response to diverse stimulants in the TME, TAMs undergo

polarized activation. The activation states of macrophages, as well as of other myeloid cells, have been defined by a nomenclature adapted from the T_H1 and T_H2 cell response, referred to as M1 (classical) or M2 (alternative) activation, respectively (Fig. **3**).

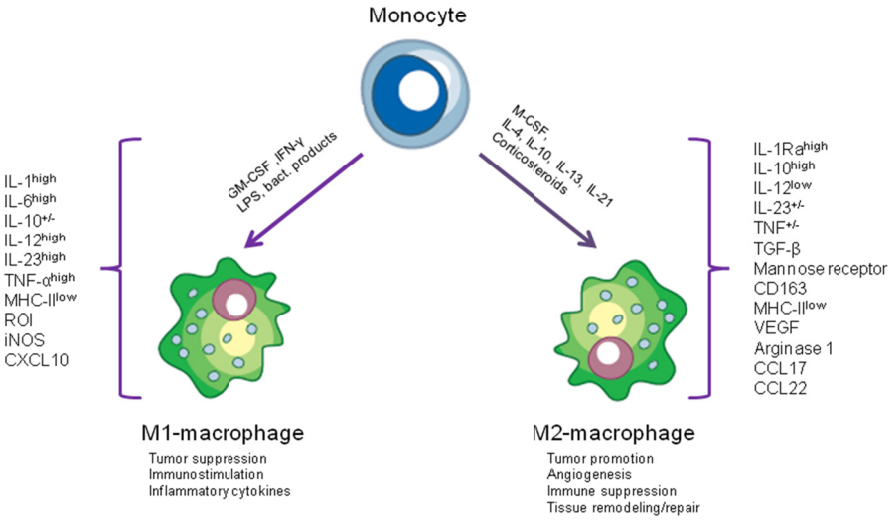

Figure 3: Tumor-associated macrophages (TAM) can either assume tumor-promoting or -suppressing functions. Monocytes are attracted to a growing tumor through a chemokine gradient and differentiate in the tumor stroma to tissue macrophages. Depending on the particular cytokine composition in the microenvironment macrophages differentiate into two major conditions, the M1- or M2-phenotype. M1-TAMs actively present tumor antigens to T cells to elicit an anti-tumor immune response. M1-macrophages also produce, among other factors, the interleukins IL-1, IL-6, IL-12 and IL-23, TNF-α and iNOS, ROI and CXCL10 that all contribute to a tumor-suppressive TME. Conversely, in a TME that contains high levels of immunosuppressive factors that promote tumor growth, such as IL-1Ra and IL-10, TGF-β and scavenger receptors (MR, CD163) as well as arginase 1, VEGF, CCL17 and CCL23, M2-macrophages assume a pro-tumor function by supplying factors that enhance tumor progression, angiogenesis, tissue remodeling and immune suppression.

The classically activated M1-macrophages are pro-inflammatory cells that, following exposure to interferon-γ (IFN-γ) or microbial products (*e.g.* LPS) release inflammatory cytokines, reactive nitrogen and oxygen intermediates, and

therefore they are endowed with an enhanced ability to kill tumor cells. In contrast, when TAMs are exposed to anti-inflammatory molecules, such as the interleukins IL-4, IL-10, IL-13 or glucocorticoid hormones and other factors, they are polarized to the opposite extreme called M2. M2-TAMs are poor antigen presenting cells and they support tumor growth, angiogenesis, and metastasis.

Conversely, TAMs suppress the immune system by responding to anti-inflammatory cytokines, apoptotic cells and immune complexes. M1 macrophage activation is characterized by high levels of major histocompatibility complex class II (MHC-II) expression and antigen presenting capacity, high production of pro-inflammatory cytokines such as IL-1, IL-12, IL-23, TNF-α and of toxic inducible nitric oxide synthase (iNOS) and reactive oxygen intermediates (ROI). In contrast, the M2 activation state is characterized by an IL-10^{high} and IL-12^{low} phenotype, expression of low levels of MHC-II and increased production of angiogenic factors and anti-inflammatory cytokines like IL-10, arginase and TGF-β. Furthermore, M1 macrophages express opsonic receptors (*e.g.* FcγRIII), whereas M2 macrophages preferentially express non-opsonic scavenger receptors such as the mannose receptor (MR) and CD163 [193-195]. In the majority of solid tumors TAMs predominantly are of the M2-phenotype. They promote angiogenesis (see Fig. **3**) and express high levels of M2-markers (IL-10, TGF-β, ARG1, CD163, MR) and low levels of mediators of inflammation (IL-6, IL-12, iNOS and TNF-α) [181, 185, 186, 196-198].

This discrimination between M1 and M2 macrophages is a rather simplified view of two extremes of polarization and it does not fully represent the continuum of functional states of macrophages in the TME. Not only the intratumoral macrophages, but also spleen and peritoneal macrophages of tumor-bearing individuals share these similar immunosuppressive properties and play an important role in tumorigenesis [199, 200]. TAMs were also shown to attract $CD4^+CD25^+FOXP3^+$ regulatory T cells [112] that are known to suppress the anti-tumor function of cytotoxic T cells. Accumulation of T_{reg} in tumors is a common feature of human cancers and the abundance, as well as their suppressor activities are highly correlated with a poor disease prognosis. In ovarian carcinoma it was found that TAMs regulate T_{reg} trafficking to tumors by producing CCL22, a chemokine that mediates regulatory T cells recruitment [201].

Numbers, polarization state and cytokine expression pattern of TAMs can be correlated in several cancer types with the clinical prognosis of the disease [38, 185, 202]. For example, high numbers of TAMs are, among others, indicative of bad prognosis in colorectal cancer [203, 204], non small cell lung cancer (NSCLC) [98, 205-207], Hodgkin's lymphoma [208], breast cancer [209, 210], liver cancer [211, 212] and prostate cancer [213].

Analysis of the molecular basis of the TAM phenotype identified components of the NF-κB signaling system as one of the main players in the modulation of macrophage function [214-216]. For example, NF-κB inhibition by targeted deletion of IKK-β in TAMs increased their anti-tumor activity through reduced production of arginase-1, IL-10 and TNF-α with concomitant increased production of iNOS and IL-12, suggesting that IKK-β signaling in macrophages maintains their alternative tumor-promoting phenotype [217]. On the contrary, in more advanced stage tumors, a therapeutic effect was achieved through the restoration of NF-κB activity in myeloid cells [218, 219]. These divergent results may be associated with progressive modulation of NF-κB activity in tumor-infiltrating macrophages. Other important modulators of macrophage polarization are members of the STAT family of transcription factors. Although earlier evidence indicated that the STAT1 activation regulates the M1 activation of macrophages, recent reports argue that activated STAT1 may induce TAM-mediated suppressive activity and tumor progression [220-222]. In addition, STAT3 and STAT6 activation were also shown to be associated with M2 macrophage polarization [223, 224]. The interplay of TAMs with immune cells (B-cells, T-cells, regulatory T-cells and neutrophils) will be described and summarized in the respective paragraphs below.

Dendritic Cells

The second cell type that belongs to the mononuclear phagocyte system (MPS) are the dendritic cells (DC). DCs are bone marrow-derived cells originating from both lymphoid and myeloid progenitors. They populate all lymphoid organs including the thymus, spleen, and lymph nodes, and comparable to the macrophages, nearly all non-lymphoid tissues and organs. DCs have potent antigen-presenting capacity for the stimulation of T cells and they also belong to

the innate immune system where they respond as immature cells to danger signals in the microenvironment by differentiating and acquiring the capacity to mount primary immune responses. DCs possess powerful adjuvant activity as they have the ability to stimulate specific CD4 and CD8 T cells [38, 180, 225-231]. This property has made them attractive targets in vaccine development strategies for the prevention and treatment of infections, allograft reactions, allergic and autoimmune diseases and cancer. A major use of DCs as immunotherapeutic vaccines consists in their *ex vivo* priming combined with adjuvant treatments that eliminate immunosuppressive mechanisms in the TME (see below).

Similar to TAMs, the dendritic cells are also infiltrating tumor tissue following chemokine signals released by the TME. These tumor-associated dendritic cells (TADC) share many properties with TAMs as they can also be polarized either to tumor-suppressive "M1-like" or to tumor-promoting "M2-like" phenotypes [38, 231-233].

Myeloid Derived Suppressor Cells (MDSC)

Myeloid derived suppressor cells (MDSCs) are another complex but well characterized population of tumor-infiltrating myeloid cells that negatively affect the anti-tumor immune response. MDSCs are a heterogeneous population of cells comprised of monocyte, granulocyte and dendritic cell precursors and myeloid cells at an early stage of differentiation [67, 234-241]. These cells are defined by the co-expression of the monocytic marker CD11b and the granulocyte differentiation antigen Gr1 (constituted by the epitopes Ly6C and Ly6G in mice). In recent studies MDSCs were broadly classified as two major subsets, namely cells of granulocytic ($CD11b^{+}Ly6G^{+}Ly6C^{low}$) and monocytic ($CD11b^{+}Ly6G^{-}Ly6C^{high}$) phenotype [242, 243].

It has been well established that the frequency of these cells significantly increases in the spleen and bone marrow of tumor-bearing mice, as well as in the peripheral blood and tumors of cancer patients [241]. In naive tumor-free mice, MDSCs constitute approximately 30% of all bone marrow cells and 3% of all nucleated splenocytes. However, in tumor bearing mice, they may represent more than 20% of all splenocytes [238]. In both patients and experimental animals,

MDSCs have been shown to be mobilized from bone marrow in response to multiple tumor-derived factors such as Bv8 and endocrine gland-derived VEGF [244, 245]. Their recruitment to tumors is mediated by chemotactic factors like CCL2/MCP-1, CXCL12/SDF-1α, CXCL5 and KIT ligand [246]. Although MDSCs are able to differentiate into mature myeloid cells upon exposure to appropriate stimuli, their differentiation is blocked by tumor cell conditioned media *in vitro* or in a tumor-bearing host *in vivo* [247]. These immature myeloid cells potently suppress maturation and anti-tumor activation of dendritic cells, T cells and natural killer cells, a phenotype that provides the most effective way of identifying MDSC [248]. Hence, injection of tumor cells in combination with $CD11b^+Gr1^+$ cells in mice prompt tumor growth [249]. Accordingly, depletion of $Gr1^+$ cells in tumor-bearing mice leads to delayed tumor growth, suggesting MDSC as potential targets for anti-cancer therapy [250-256]. A report by Youn and colleagues indicated that $CD11b^+Gr1^+$ cells from naïve tumor-free mice are not immune suppressive [243]. However, it is not yet fully known why $CD11b^+Gr1^+$ cells isolated from tumor-free and tumor-bearing animals exhibit different functions. A recent study suggested a HIF-1α mediated regulatory mechanism for the biological dichotomy displayed by MDSCs within the TME. These researchers demonstrated that splenic MDSCs of tumor bearing animals cause ROS mediated antigen-specific T cell unresponsiveness, whereas intratumoral MDSCs with similar morphology and phenotype suppress both antigen specific and nonspecific T cell function through elevated NO levels and arginase I production [257].

Neutrophils

Neutrophils are short-lived white blood cells derived from bone marrow myeloid progenitors. During infection-related immune responses neutrophils are among the first cells to arrive at the site of infection where they release chemokines and proteases that trigger the recruitment of both innate and adaptive immune effector cells. Neutrophils also release cytotoxic mediators, including reactive oxygen species, membrane-perforating agents, proteases and soluble mediators such as interferons, TNF-α and IL-1β, suggesting their potential anti-tumor activity [72, 258, 259]. Generally, in most tumors low numbers of neutrophils are found. Both

cancer cells and cells of the TME actively recruit neutrophils by means of secreted chemotactic factors, in particular G-CSF, GM-CSF, CXCL2/MIP-2α, CCL3/MIP-1α, CXCL5/LIX and CXCL1/KC. Upon recruitment to the tumor site, neutrophils can assume tumor growth-stimulatory or -inhibitory functions [71]. In human tumors, an increased density of tumor-infiltrating neutrophils was found to correlate with a poor prognosis in patients with adenocarcinoma and metastatic melanoma, whereas in few cases like gastric carcinoma neutrophil infiltration was linked to beneficial disease outcome [260-262]. This discrepancy is probably related with the degree of neutrophil recruitment and their differential activation, depending on the intratumoral cytokine microenvironment in which they reside. Similar to TAMs, the functional status of tumor associated neutrophils (TANs) regulates their ability to express an anti-tumor potential. Accumulating experimental and clinical evidence also confirms that neutrophils can polarize in a type I or type II direction in tumors. Recently, Fridlender and colleagues characterized N1- and N2-polarized phenotypes of TANs, similar as described for TAMs [263]. In lung and mesothelioma tumor models, TANs were shown to acquire a N2-phenotype. The pro-tumorigenic activities of N2-TANs include increased production of immunosuppressive cytokines and reduced cytotoxic activity. This pro-tumor phenotype of neutrophils was found to be induced and maintained by TGF-β [264]. N1-polarized neutrophils exert anti-tumor activities indirectly as well by promoting recruitment and activation of $CD8^+$ T cells. In addition to induction of the anti-tumor N1-polarization, blocking of the TGF-β pathway caused increased recruitment of $Ly6G^+$ neutrophils in tumors [263]. This finding is consistent with studies that demonstrated an enhanced influx of myeloid cells into mammary carcinomas deficient in type-II TGF-β receptor [249]. Further, it was shown that abrogation of TGF-β signaling in human breast cancer cells enhanced the production of the neutrophil chemoattractants CXCL1 and CXCL5 [265]. Apparently, TGF-β is one of the major players in regulating neutrophil recruitment and activation in the TME. A recent study suggested that constitutive expression of IFN-β counteracts the cancer-supportive function of neutrophils by inhibiting expression of genes encoding pro-angiogenic and homing factors in these cells [266].

In summary, as shown in Fig. **4**, the types of cells infiltrating a tumor microenvironment and their state of polarization control the fate of a growing tumor. Type-1 polarized macrophages and neutrophils, mature DCs and mature T cells with T_H1 activity create a tumor growth inhibitory environment. At the opposite, type-2 polarized macrophages and neutrophils, immature DCs, MDSC, regulatory T cells and T_H2 T cells promote angiogenesis and tumor growth.

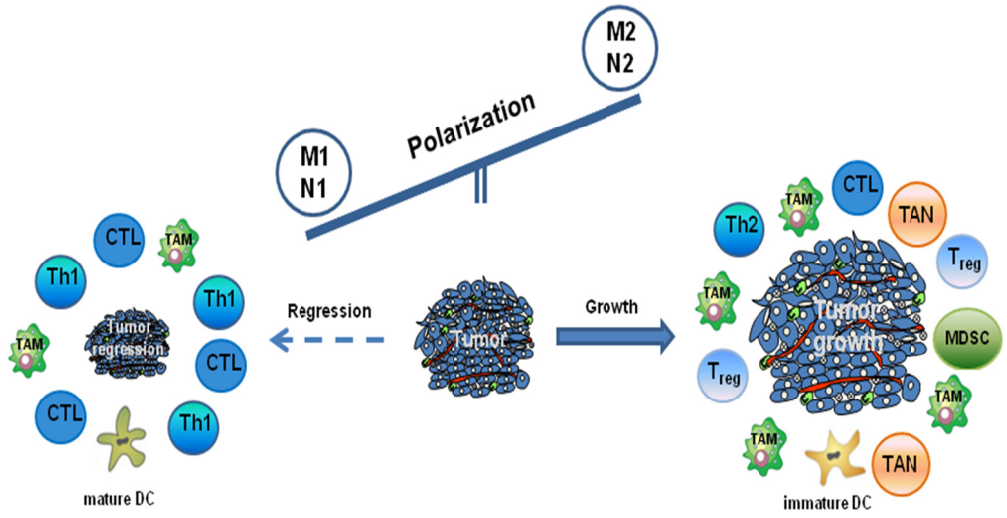

Figure 4: Immune cells infiltrating a tumor regulate tumor growth, progression and metastatic dissemination. Depending on the state of polarization of tumor associated immune cells tumor development is suppressed or enhanced. Tumor regression is associated with M1-macrophages, N1-neutrophils, mature dendritic cells (DCs) and mature T cells with T_H1-activity. In contrast, tumor growth is facilitated *via* immune-suppression and induced angiogenesis, M2-macrophages, N2-neutrophils, immature DCs and plasmacytoid DCs, myeloid-derived suppressor cells (MDSCs), regulatory T cells and a low frequency of T_H2 activated CD4 and CD8 effector T cells.

Therapies Aiming at Components of the TME

Cell-based Therapies

Based on a vast amount of clinical and pre-clinical evidence, our current knowledge suggests that therapeutic targeting should not only be aimed at the malignant cancer cells, but also at the components of the TME to effectively inhibit tumor growth. Thus, interference with microenvironmental growth support is becoming appreciated as an attractive therapeutic strategy [267, 268]. As a key

component of the TME, the tumor promoting properties of myeloid cells render these cell types as valuable tools and targets for therapeutic interventions. One of the first strategies that have been explored since many years is the adoptive immunotherapy which consists in the transfusion of host derived and *in vitro* activated or engineered lymphoid cells. Transfer of tumor infiltrating leukocytes (TIL) to tumor bearing hosts mediates antitumor responses and several myeloid cell subpopulations were found to be suitable for use in adoptive immunotherapy. Lymphocytes treated with IL-2 give rise to lymphokine activated killer (LAK) cells that have the ability to lyse malignant but not normal cells. Clinical studies in patients with advanced cancer revealed that treatment with IL-2 alone or in combination with LAK cells mediate complete or partial regression of cancer, predominantly melanomas [269-273]. Other methodologies either used combinations of lymphokines, such as TNF-α or interferons in conjunction with IL-2 or gene therapy approaches to further improve the effects of adaptive immunotherapy [274-278]. Although the significance of MHC class I-restricted cytotoxic T lymphocytes (CTLs) as effectors of anti-tumor immunity has widely been demonstrated, most human tumors lack MHC-I expression or are inadequately differentiated and poorly immunogenic, a culprit that limits successful T-cell based tumor-specific immunotherapy [279]. In another cell-based therapy approach efficient tumor-specific effector and memory T cells are induced through therapeutic vaccination. Such vaccines follow two purposes, namely priming antigen-specific T cells and reprogramming memory T cells by transforming them from the immunosuppressive to the immunostimulating and cytotoxic phenotype. Dendritic cells (DCs) are very potent antigen presenting cells and thus essential in generation of immune responses, and they therefore represent valuable targets and vectors for cancer vaccination [280-293].

Therapies Aimed at TAMs

Based on the M1 *versus* M2 paradigm of macrophage polarization, inhibition of M2- and activation of M1-inducing signals was suggested as a potential strategy to re-establish the anti-tumor function of macrophages [294]. Indeed, pharmacological skewing of macrophage polarization from the M2- to M1-phenotype is able to induce an anti-tumor activity. Co-administration of the macrophage chemoattractant CCL16 with a CpG oligonucleotide and an anti-IL-

10 receptor antibody was shown to skew M2-TAMs to M1-TAMs that triggered an innate response resulting in the regression of pre-established tumors [295]. Similarly, combination of an anti-CD40 antibody with CpG oligonucleotides and multidrug chemotherapy induced antitumor effects by TAM polarization [296]. Considering the central role the statins play in myeloid cell polarization, members of the STAT family of transcription factors are valuable targets for the modulation of myeloid cells. To this end, tumor bearing $STAT6^{-/-}$ mice were shown to display an M1-TAM phenotype and to reject a spontaneously growing mammary carcinoma [297, 298]. Accordingly, it was found that the SHIP1 phosphatase plays an important role in macrophage re-programming. Mice deficient in SHIP1 displayed a skewed development toward M2-TAM and thus pharmacological modulators of this phosphatase could be developed [299]. More recently, a host-derived factor, histidine-rich glycoprotein (HRG) was reported to promote M1 polarization of TAMs by downregulation of PLGF [300].

Other approaches aim at the depletion of TAMs, either by blocking vital functions of the cells or by their physical depletion. In various models it was shown that blockade of the macrophage specific colony-stimulating factor 1 (CSF-1) or its receptor CSF-1R suppresses macrophage infiltration and tumor growth [301-303].

The physical (pharmacological) depletion of macrophages from organs of the MPS using the bisphosphonate clodronate encapsulated in liposomes (Clodrolip) has become an important, reliable and widely used method to study not only the role of macrophages in the immune system and in inflammatory processes but also in tumor growth and metastasis [304-311].

Bisphosphonates

Bisphosphonates (BPs) are inorganic pyrophosphate analogs (PPi) that effectively inhibit osteoclastic bone resorption and are widely used to treat metabolic bone diseases, such as postmenopausal osteoporosis [312], Paget's disease [313], tumor associated osteolysis [314] and to prevent bone metastasis [315]. The high affinity of the BPs for the calcium component of the bone matrix hydroxyapatite is the cause of the bone-specificity of these compounds. Organ distribution studies demonstrated that BPs are mainly localized in newly formed bones and internalized by the bone resorbing osteoclasts where they inhibit their activity [316]. Due to their high affinity

for bone matrix, systemic availability of BPs is rather low with the exception of a transient raise of plasma levels in the post-administration period [317].

Based on their chemical structure BPs can be divided into two distinct pharmacological classes; the nitrogen-containing bisphosphonates, (N-BPs, *e.g.* zoledronate) and the first-generation BPs, the non-nitrogen-containing bisphosphonates (non-N-BPs, *e.g.* clodronate, see Fig. **5**) that chemically resemble pyrophosphate (PPi). Pyrophosphate has a P–O–P structure, whereas the BPs have a P–C–P structure where the central oxygen atom is replaced by a carbon atom. The most important first generation non-N-BP bisphosphonate, clodronate has R^1 and R^2 side chains with two chlorine atoms, whereas the N-BP zoledronate carries a hydroxyl group on R^1 and an imidazolyl group on R^2. BPs containing nitrogen atoms in the R^2 side-chain like zoledronate are significantly more potent than non-N-BPs [317]. The mechanism of action of the BPs differs according to their chemical structure. After cellular uptake, non-N-BPs are metabolized to cytotoxic analogs of adenosine triphosphate (ATP) causing cell death by apoptosis [318]. The N-BPs exert their effects mainly by inhibiting a key enzyme in the mevalonate pathway, the farnesyl pyrophosphate synthase (FPP synthase), thereby preventing the synthesis of isoprenoid compounds that are essential for the post-translational modification of small guanosine triphosphate (GTP)-binding proteins such as Rab, Rho, and Rac [319]. Recent studies revealed that N-BPs can also induce formation of a new pro-apoptotic ATP analog that induces mitochondria-mediated apoptosis [320].

Although the most effective N-BP zoledronate has originally been developed to inhibit osteoclast mediated bone resorption, the anti-cancer effects of this compound are currently being evaluated. In this context zoledronate is used as adjuvant therapy to inhibit local bone destruction by tumors and to prevent or delay metastasis to bone [321-323]. Moreover, zoledronate has demonstrated significant clinical benefits in patients with metastatic prostate and lung cancer [322, 324, 325]. Zoledronate exerts these anti-tumorigenic activities directly on cancer cells by modulating their tumorigenic properties and indirectly on stromal cells by changing their tumor-promoting properties. One of the major anti-tumor effects of zoledronate is the induction of apoptosis but the drug also interferes with migratory and invasive properties of tumor cells [326-329] and with angiogenesis [330-332]. In addition to

its pharmacological effects, zoledronate has immune modulatory activities that include stimulation of proliferation and activation of the Vγ9Vδ2 subset of γδT cells. T cells expressing the Vγ9Vδ2 T cell receptor play a significant role in immune surveillance and defense [333-335]. These cells have the ability to recognize and kill tumor cells in an MHC-independent manner, suggesting their potential utility in the elimination of cancer cells with poor antigen presentation capacity [336]. Several pre-clinical studies have shown that Vγ9Vδ2 T cells expanded *in vitro* sustain their anti-cancer activity upon adoptive transfer into mice transplanted with various human cancer cells along with zoledronate treatment [337-340]. Clinical studies also demonstrated expansion and activation of Vγ9Vδ2 T cells to a subset of IFN-γ producing effector T cells in patients treated with zoledronate, either alone or in combination with IL-2 [337, 341, 342]. Besides cancer cells, monocytes treated with zoledronate were also shown to stimulate proliferation and cytotoxic activation of human Vγ9Vδ2 T cells. Notably, activation of γδT cells requires cell-to-cell contact with zoledronate treated tumor cells or monocytes [343]. Among several growth factors, TGF-β is known as the most abundant cytokine in bone and considered as the main bone-derived factor responsible for driving this vicious cycle of bone metastasis [344]. Activated TGF-β is released from mineralized bone matrix and in turn it induces production of tumor-derived osteolytic factors [345-349].

These and other data suggest that modulation of bone derived factors like TGF-β might also be a possible mechanism responsible for the anti-tumor activity of zoledronate. Altogether, pre-clinical and clinical studies suggest multifaceted anti-cancer effects of zoledronate in different tumor types. In addition, clinical studies showed that zoledronate prolongs disease-free survival in cancer patients [350]. However, the identification of new cellular targets and further elucidation of the cellular and molecular mechanisms by which zoledronate mediates anti-tumor effects will be useful in the design of new therapeutic strategies to modulate and potentiate the anti-tumor effects of this compound.

Depletion of TAMs with Clodronate-Liposomes (Clodrolip)

Exploiting the anti-tumor properties of the bisphosphonates and in particular clodronate-liposomes, we examined the possibility whether depletion of TAMs would inhibit tumor angiogenesis and tumor growth. In our experiments, we showed for the first time that treatment of tumor bearing mice with Clodrolip as

single therapy in comparison to free clodronate and in combination with anti-VEGF single chain fragment antibodies, resulted in drastic tumor growth inhibition and exhaustion of TAM cell populations [351] (Fig. **5**).

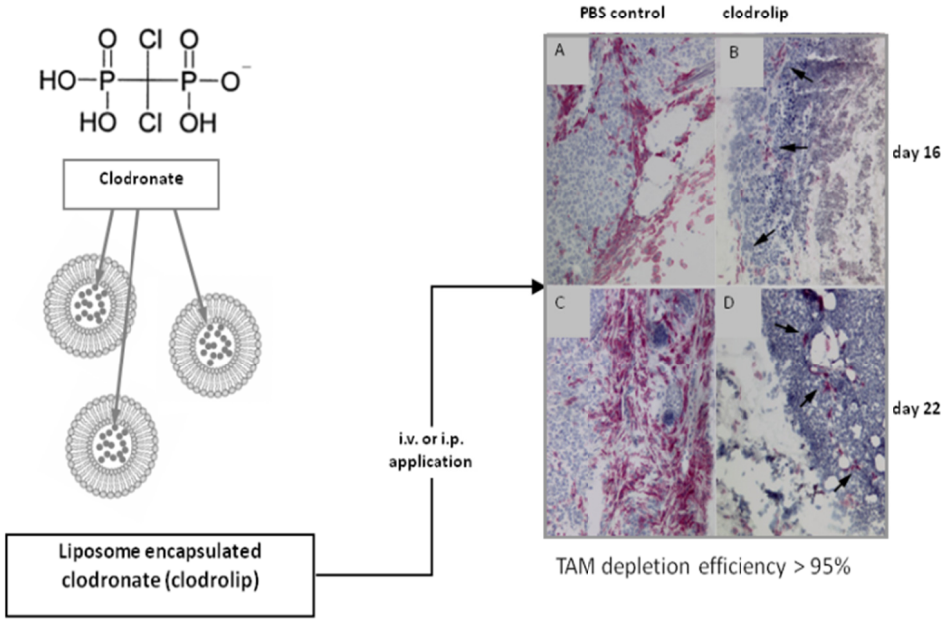

Figure 5: Scheme of clodronate and encapsulated clodronate (Clodrolip) in small unilamellar liposomes. Example of macrophage depletion efficiency of Clodrolip given by the i.p. or i.v. route in A673 rhabdomyosarcoma tumors and analyzed by immunohistochemistry at day16 and 22 after tumor cell inoculation. Cells stained in red are $F4/80^+$ positive TAM. Adapted from [351].

Based on our findings, summarized in Table **1**, several follow-up studies using Clodrolip: or other clodronate-liposomes confirmed the therapeutic validity of the TAM depletion method. In fact, clodronate- or other bisphosphonate liposome-mediated macrophage depletion or modulation opens new opportunities to study the role of tumor infiltrating cells and combined with anti-angiogenic or cytotoxic therapies TAM depletion represents a promising new approach of high clinical potential.

Table 1: Effects on Tumor Growth by Clodrolip mediated Depletion of TAMs in select preclinical Tumor Models

Models (Tumor cells, treatments)	Effects of TAM Depletion	Notes	Refs.
Breast cancer (MDA-MD-231, MVT-1) overexpr. S100A7	Inhibition of the effects of S100A7 induction on tumor growth and angiogenesis in orthotopic models.	S100A7 is overexpressed in invasive estrogen receptor α-negative breast cancer and activates pro-inflammatory pathways.	[363]
F9 teratocarcinoma in Sv129 mice	Depletion correlated positively between TAM-densities and mesenchymal marker expression.	TAMs induce EMT through TGF-β signaling and β-catenin activation. Clinical relevance is shown in non-small cell lung cancer (NSCLC).	[98]
Lung cancer induced by urethane in FVB mice	Alveolar macrophage depletion reduced number and size of lung tumors and inhibited angiogenesis.	Urethane treatment induced M1 macrophages (first 2-3 wks) followed by M2 macrophages by week 6.	[364]
Bladder cancer (MBT-2)	TAM depletion by Clodrolip or VEGF block inhibited lymphangiogenesis and lymph node metastases but not growth of orthotopic primary tumors.	Massive lymphangiogenesis and TAM infiltration in primary tumor and metastasis in lymph nodes.	[365]
Liver cancer (Hepa 1-6)	TAM depletion reduced tumor growth in s.c. and othotopic liver tumors.	TAMs express MHC-II^{high} at early stages and pro-tumorigenic MHC-II^{low} during tumor growth.	[366]
Hepatocellular cancer xenografts, sorafenib treatm.	TAM depletion or zoledronate (zol) + sorafenib inhibited tumor progression, angiogenesis and lung metastasis.	Combined therapy with zol or TAM depletion enhanced the effect of sorafenib. Zol was more effective than Clodrolip.	[354]
Melanomas in C57BL/6 and TNFR1,2$^{-/-}$, TNF$^{-/-}$ mice, local radiation therapy	TAM depletion before radiotherapy increased antitumor effects of ionizing radiation in a TNFα dependent way.	Treatment with a TNF receptor fusion protein (Enbrel) showed that macrophage mediated radioresistance required intact TNFα signaling.	[367]
Colon adenocarcinoma (MC38), mammary tumors (AT-3, 4T1.2) targeting DR4 and DR5 with mab MD5-1	MD5-1 mab treatment inhibited tumor growth by TRAIL-R dependent tumor cell apoptosis. Clodrolip treat-ment enhanced efficacy of MD5-1.	Ab-mediated targeting of DR5 triggers tumor cell apoptosis in a B cell-dependent manner. Contribution of NK cells, CD11b^{+} cells, and macrophages to the antitumor effects of MD5-1.	[368]
Colon adenocarcinoma (MC38), renal cell carcinoma (Renca) combination of CpG 1826 with a CD137 specific T-cell antibody	CpG plus anti-CD137 caused tumor regression. TAM depletion enhanced therapy leading to tumor rejection in 100% of mice.	This study provides support for the use of a novel combination of immunomodulatory agents stimulating multiple facets of immunity for the effective immunotherapy of cancer.	[369]
HPV16 E6- and E7-expressing TC-1 mouse tumor model	TAM depletion inhibited tumor growth and stimulated HPV16 tumor infiltration by virus-specific CD8 lymphocytes.	M2-like macrophages infiltrate HPV16-associated tumors causing suppression of antitumor T-cell response.	[370]

Table 1: contd……

Ovarian carcinoma (MDAH-2774, SKOV-3, OVCAR3) in nude mice	Depletion of macrophages by Clodrolip markedly reduced lymph-angiogenesis.	Blockade of VEGF/VEGFR signaling or depletion of macrophages reduced lymph-angiogenesis.	[371]
Lung cancer (HARA-B) injected into the left cardiac ventricle of mice	Clodrolip significantly reduced the number of macrophages in tumors and osteoclasts in bone marrow.	Clodrolip exerted antimetastatic effects in both bone and muscle.	[372]
Rat glioma (D74/HveC), oncolytic viruses (OV) injected into intracranial gliomas	Depletion of TAMs enhanced intratumoral OV spread.	$CD163^+$ macrophages infiltrated the tumor. TAM depletion during OV delivery helps intratumoral propagation and persistence of virus, rendering more efficient therapy.	[373]
Murine teratocarcinoma (F9) and human rhabdomyo-sarcoma (A673)	75 - >92% TAM depletion with Clodrolip. Combination therapy of Clodrolip plus a VEGF-neutralizing antibody was most effective.	First demonstration of TAM depletion. Tumor inhibition was accompanied by drastic anti-angiogenic effects. $CD11c^+$ TADCs were also depleted by Clodrolip or antibody treatment.	[351]

Zoledronate has also been encapsulated in liposomes either targeted to the folate receptor expressed on tumor cells showing cytotoxic activity [352] or in (polyethylene)glycol liposomes tested in murine models of human prostate cancer and multiple myeloma where the liposomal formulation proved to be more cytotoxic compared to the free drug [353]. Another study showed that treatment of hepatocellular carcinoma xenografts with sorafenib, a multikinase inhibitor, was markedly enhanced by concomitant depletion of macrophages by clodrolip or free zoledronate [354].

Drug formulations with liposomes are also used to target other cell types in the TME including the tumor cells themselves [355-357]. The high vascular permeability of tumor blood vessels can be exploited for increased accumulation and retention of macromolecules and liposomes in the tumor tissue. Passive targeting of long circulating liposomes to tumors with liposomal doxorubicin was one of the first clinically approved drug application with enhanced activity and reduced toxicity [358] and several other drugs are currently being evaluated as liposome formulations.

Extravasation and accumulation of liposomal drugs within the TME occurs because small liposomes are able to penetrate through the leaky vasculature into

the tumor tissue where they are taken up by cells such as macrophages or dendritic cells or where they release the encapsulated payload into the ECM. In an earlier mouse tumor model study, we demonstrated the specificity and cytotoxicity of immunoliposomes that were targeted against the ED-B isoform of fibronectin which is uniquely expressed in the ECM of solid tumors [359].

Other examples of target-specific immunoliposomes are doxorubicin loaded anti-HER2 immunoliposomes [360] or epidermal growth factor receptor (EGFR)-targeted immunoliposomes [361]. In summary, nanocarriers, most notably liposomes, possess a great potential for the delivery of cytotoxic drugs or immunomodulating agents to the TME and to metastases [362].

Therapies Aimed at TANs

To date, the anti-tumor potential of neutrophils has received scarce attention and their functions as effective weapons against cancer are still not fully exploited. Yet, recently gathered evidence indicates that under appropriate stimulation neutrophils reveal very powerful tumor-inhibitory properties. As neutrophils in tumor-bearing hosts have impaired cytotoxic activity, the development of methods that stimulate recruitment and anti-tumorigenic activation within a TME can be exploited as new therapeutic opportunities.

Early studies with cytokine or chemokine gene transfected mammary adenocarcinomas in syngeneic tumor models indicated that nonspecific mechanisms, mostly supported by neutrophil functions, had much greater therapeutic power than those elicited by specific immunity [374-376]. For example, local or systemic administration of rIL-12 in mice bearing subcutaneous mammary carcinoma resulted in a rapid influx of neutrophils with high cytotoxic potential and anti-angiogenic function [377]. TGF-β has been defined as a major functional regulator of neutrophils. Specifically in tumors, TGF-β has been found to drive the pro-tumorigenic polarization of neutrophils. Thus, inhibition of TGF-β signaling offers a means to manipulate neutrophil polarization by shifting N2-TANs to tumor growth inhibiting N1-neutrophils. Additionally**,** TGF-β receptor blockage in tumor bearing mice was shown to induce the activation of $CD11b^{+}Ly6G^{+}$ neutrophils that resulted in a significant tumor growth delay [263].

Therapies Aimed at CAFs and Mast Cells

CAFs represent another therapeutic target within the TME. However, due to a lack of compounds that specifically target this cell population, such strategies have not been widely used in the clinical setting. The most studied target molecule is the fibroblast activation protein (FAP) that is selectively expressed on stromal fibroblasts or on CAFs. FAP is a membrane-bound serine protease of the prolyl oligopeptidase family with distinctive endopeptidase activity and with low or undetectable expression in fibroblasts of normal tissues [378, 379]. In a preclinical vaccine approach, it was shown that immunological targeting of FAP can elicit protective immunity. A DNA vaccine directed against FAP suppressed primary tumor growth and pulmonary metastases primarily through $CD8^+$ T-cell-mediated killing in tumor-bearing mice [380].

Mast cells (MC) play an essential role as effector cells in allergy but they also contribute to tumor development. Activated MC located in the TME release angiogenic and tumor growth stimulating factors [124, 125, 381]. Recent findings indicate that tumor-associated mast cells might represent valuable targets for therapeutic interventions, most notably to kinase inhibitors as c-Kit [128, 382].

Other Therapies: Anti-angiogenic Therapies, Antibodies, Antibody-drug Conjugates, Cytokines, Gene Therapeutics

The major non-cellular therapies aiming at specific targets in the TME including antibodies and small molecule inhibitors are summarized in the following section.

Anti-Angiogenic Therapies

The vascular endothelial growth factor (VEGF) proteins are key regulators of normal and tumor angiogenesis and they are therefore extensively studied as therapeutic targets [383]. Antibodies and fusion proteins targeting VEGF are the clinically approved bevacizumab (Avastin, Genentech) [384-386], r84 (AT001, Affitech AS), a human antibody which inhibits VEGF from binding to the VEGF-receptor-2 and VEGF-trap which is a fusion protein containing the binding domains of the VEGF-receptors 1 and 2 fused to the human IgG Fc region [383]. However, anti-angiogenic therapies may be compromised by the finding that myeloid $CD11b^+Gr1^+$ cells which contribute to tumor angiogenesis render tumors

refractory to angiogenic blockade by VEGF antibodies. This $CD11b^{+}Gr1^{+}$-mediated effect is driven by the protein Bv8 which, in turn, is up-regulated by G-CSF. Thus, G-CSF may contribute to tumor refractoriness to anti-angiogenic therapies [66, 245, 387]. Different anti-angiogenic compounds such as small molecule tyrosine kinase inhibitors TKIs (*e.g.* sunitinib, sorafenib, imatinib, dasatinib, nilotinib and the proteasome inhibitor bortezomib) and other immunomodulatory drugs targeting VEGF or other pathways seem to be capable of modulating immune responses, in a positive as well as a harmful manner. Recent studies focused not only on their direct anti-tumor responses, but also on their influence on the TME, as well as on their effects on malignant and healthy cells. Thus, for an optimal clinical anti-cancer treatment, a better understanding of these immunomodulatory effects is essential [388].

Unfortunately, the initial expectations and optimism for therapies with anti-angiogenic drugs targeting the VEGF signaling pathway were impeded by the limited clinical benefits. New data indicate that the unique characteristics of the tumor vasculature within the TME may hold the key for successful novel anti-angiogenic therapies. The molecular and cellular alterations that maintain aberrant tumor angiogenesis represents novel targets for improving current anti-angiogenic strategies [389]. This so-called "vascular normalization" is characterized by attenuation of hyperpermeability, increased vascular pericyte coverage and a normalized basement membrane, resulting in the reduction of tumor hypoxia and interstitial fluid pressure. This improves the metabolic profile of the TME and the delivery and efficacy of therapeutics. Novel genetic and pharmacological approaches characterized key regulators of vascular normalization such as proteins that regulate tissue oxygen sensing and vessel maturation [45, 390-392].

Antibodies

Monoclonal antibodies have considerably modified the therapy concepts in clinical oncology. Antibodies and smaller fragments such as antigen-binding fragments (Fab), single chain variable fragments (scFv) and smaller molecules are produced by recombinant technologies [393]. Antibodies possess several clinically relevant mechanisms of action. They can manipulate tumor-related signaling and various antibodies show immunomodulatory properties and, by

activation or inhibition of the immune system, they can induce antitumor immune responses [394, 395]. Specifically, Fc-receptor expressing immune cells mediate the killing of tumor cells by mAbs. Stimulation of these immune effector cells therefore represents an interesting strategy to improve the therapeutic efficacy of mAbs. The stimulation of natural killer cells, γδT cells, macrophages, or dendritic cells can be used to enhance antibody-dependent cellular cytotoxicity, phagocytosis or tumor vaccine effects [396]. Besides supporting development and strengthening of the adaptive immunity, therapeutic antibodies are able to trigger early anti-tumor events such as receptor blockade, cytostasis, apoptosis, complement-dependent cytotoxicity and/or antibody-dependent cytotoxicity [397-399]. Bispecific antibodies are used to mount and sustain tumor-specific cellular responses or in radioimmunotherapy to improve target binding, selectivity, and efficacy [400-403]. A widely studied target is the cytotoxic T-lymphocyte-associated antigen CTLA-4, also called CD152, which regulates T-cell activation. Antibodies that block the interaction of CTLA-4 with its ligands B7.1 and B7.2 enhance immune responses, including antitumor immunity. The recently FDA-approved anti-CTLA-4 antibody ipilimumab (Yervoy) and tremelimumab are the most advanced antibodies for the treatment of metastatic melanoma [404-408].

Antibody-Drug Conjugates

The development of antibody-drug or antibody-enzyme immunoconjugates for a more specifically targeted and efficient delivery of active compounds to target tumor cells has been followed since more than three decades. Several immunoconjugates, particularly those that incorporate internalizing antibodies and tumor-selective linkers have demonstrated impressive activity in preclinical models. Immunoconjugates that deliver doxorubicin, maytansine and calicheamicin were among the first to be evaluated in clinical trials [409,410]. The immunoconjugate gemtuzumab ozogamicin (Mylotarg, CMA-676), a calicheamicin conjugate that targets CD33, has been approved by the Food and Drug Administration (FDA) in 2000 for treatment of acute myelogenous leukemia (AML). Although gemtuzumab ozogamicin improved survival in a subset of AML patients when combined with standard chemotherapy, the drug was recently withdrawn by the FDA due to safety concerns [411]. However, the cytotoxic activity of the immunoconjugate confirms that CD33 remains a possible

therapeutic target for AML. In recent years, significant progress owing to the optimization of several parameters, including mAb specificity, drug potency, linker technology, and the stoichiometry and molecular sites of attachment of conjugated drugs has been made. These developments have led to an increase of conjugates being tested clinically, three of which are currently in late stage clinical trials: brentuximab vedotin (SGN-35) for Hodgkin lymphoma; trastuzumab-DM1 for breast cancer and inotuzumab ozogamicin for non-Hodgkin lymphoma [412]. The immunoconjugate trastuzumab emtansine (T-DM1) is a tumor-activated prodrug resulting from the conjugation of the cytotoxic and antimitotic maytansine derivative DM1 with the humanized anti-HER2 mAb trastuzumab which has been used for the treatment of breast cancer for over 10 years. The maytansinoids bind microtubules in a manner similar to the vinca alkaloids, but they block mitosis 20 to 100-fold more potently. Clinically, trastuzumab emtansine exhibited efficacy in patients with $HER2^+$ metastatic breast cancer. Furthermore, preclinical studies have reported that trastuzumab emtansine potentiates the effect of several chemotherapeutic agents (carboplatin, 5-fluorouracil and docetaxel), other antibodies as well as receptor tyrosine kinase and PI3K inhibitors. Many of these combinations are currently investigated in humans [413]. Phase I and II trials of T-DM1 as single agent and in combination with paclitaxel, docetaxel and pertuzumab have shown clinical activity and favorable safety profiles in $HER2^+$ metastatic breast cancer patients. Additional combinations of T-DM1 with antitumor drugs and additional disease settings such as early-stage $HER2^+$ breast cancer are also under investigation [414, 415]. Brentuximab vedotin (SGN-35) is a novel antibody-drug conjugate consisting of the anti-CD30 antibody cAC10 conjugated by a protease-cleavable linker to monomethyl-auristatin E, a potent microtubule blocking agent. In phase II trials, response rates of 75% in relapsed/refractory Hodgkin's lymphoma and 87% in relapsed/refractory systemic anaplastic large-cell lymphoma were recently reported. The impressive response rates and limited toxicity of brentuximab vedotin (SGN-35) are very promising for relapsed/refractory patients with few treatment options. In 2011, brentuximab vedotin was approved in the US for the treatment of Hodgkin lymphoma after failure of autologous stem cell transplant (ASCT) or after failure of multiagent chemotherapy regimens in ASCT-ineligible candidates and for the treatment of systemic anaplastic large-cell lymphoma after

failure of prior multiagent chemotherapy regimens [416]. The efficacy of brentuximab vedotin in other CD30 positive lymphomas is currently under investigation [417-419].

Radioimmunotherapy (RAIT) of non-Hodgkin lymphoma (NHL), a disease that is radiosensitive as well as readily accessible to the antibody conjugates using directly labeled MAbs is of current interest after approval of the radiolabeled anti-CD20 MAbs ^{131}I-tositumomab and ^{90}Y-ibritumomab tiuxetan [420]. The high efficacy of RAIT was illustrated with the nearly 100% overall response rate obtained in a clinical trial using an investigational radiolabeled anti-CD22 MAb, ^{90}Y-epratuzumab. The advantage of pretargeted RAIT over directly labeled MAbs is continuing to be validated in preclinical models of lymphomas and solid tumors. The advantages of combining RAIT with radiation sensitizers, with immunotherapy or drug conjugates targeting different antigens are being studied clinically and preclinically [421].

Comprehensive and updated lists of therapeutic antibodies and conjugates including their status of clinical use can be found at the website of the international ImMunoGeneTics information system (IMGT) (http://www.imgt.org/mAb-DB/index) and in the “Marketed therapeutic antibodies compendium” [422].

Antibody-enzyme conjugates are directed at tumor-associated antigens to achieve site-specific activation of prodrugs to potent cytotoxic drugs. This "antibody-directed enzyme prodrug therapy" (ADEPT) technology has attracted considerable interest since the concept was first described in 1987 [423]. A particular advantage of the ADEPT approach is that it may allow the use of extremely toxic and potent agents at very low concentrations. The principle of ADEPT therapy is to use a tumor-associated antigen specific antibody to target an enzyme to tumor cells. The enzyme should be retained in the tumor after clearance from blood and normal tissues. A nontoxic prodrug, which is a substrate for the enzyme is then applied and by cleaving of the enzyme-prodrug complex a potent cytotoxic agent is generated in the tumor tissue [424-426]. More recently, complementing the ADEPT technology, the promising approaches GDEPT (gene-directed enzyme prodrug therapy) and PMT (prodrug monotherapy) have been

developed. GDEPT and PMT allow a selective release of cytotoxic agents from non-toxic prodrugs at the tumor site either by enzyme encoding genes or by exploiting physiological and metabolic aberrations in cancerous tissue [427].

Chemokines and Cytokines

As mentioned, the TME contains chemokines and cytokines which are vital factors for the regulation of tumor growth, invasion and metastasis. Beyond activating tumor vascularization, infiltrating myeloid cells also promote tumor growth by creating a microenvironment that is rich in growth factors and pro-inflammatory cytokines that stimulate proliferation and survival of neoplastic cells [26, 108, 428]. Chemokines/cytokines and their receptors represent potential targets for therapeutic intervention, either with antibodies or small molecule antagonists. On the other hand, due to the complexity of the TME, and the large number of chemokines/cytokines and receptors that are also expressed by normal cells, issues remain regarding the targetability of inhibitors and whether the redundancy of the system will compensate an inactivated chemokine/cytokine or its receptor [429, 430].

The most studied cytokines for cancer immunotherapy are the interleukins (IL). *Ex vivo* treatment of lymphocytes with IL-2 gives rise to lymphokine activated killer (LAK) cells and clinical studies in patients with advanced cancer showed that treatment with IL-2 alone or in combination with LAK cells mediate complete or partial regression of cancer, predominantly melanomas and renal cell carcinoma [269-273, 431]. More recently, several new interleukins, namely IL-12 in ovarian cancer [432, 433], IL-15 in various experimental tumor models [434], IL-18 in metastatic melanoma [435] and IL-21 in early phase renal cell carcinoma and melanoma clinical trials [436] have been characterized that have considerable promise for future immunotherapy [437]. IL-15 binds to its specific receptor, IL-15Rα, which is expressed on dendritic cells, monocytes and macrophages. IL-15 induces differentiation and proliferation of T, B and natural killer cells. It also enhances the cytolytic activity of $CD8^+$ T cells and induces $CD8^+CD44^{high}$ memory T cells. Furthermore, IL-15 stimulates cell differentiation and immunoglobulin synthesis by B cells and induces maturation of dendritic cells [438]. IL-18 functions mainly as a co-stimulatory cytokine and its optimal

efficacy may be obtained in combination with other immunostimulatory therapeutics. Finally, IL-27 which is a member of the IL-6/IL-12 heterodimeric cytokine family acts on naive $CD4^+$ T cells and plays pivotal roles as a proinflammatory cytokine and generation of CTLs. Recent studies revealed that IL-27 plays an important role in $CD8^+$ T cells as well [439].

Lastly, the interferons (IFN) are cytokines with a long history of use as immunotherapeutic drugs. The initial use of interferons in cancer therapy was based on their growth inhibitory and immunomodulatory effects, and more recently they have been shown to possess cytotoxic and anti-angiogenic properties. However, the availability of novel alternative therapies have replaced IFN therapy in many cancers [440]. Interferon-α (IFN-α) is a type-I interferon which exerts multiple biological effects, including antiviral and antitumor activities in patients with defined types of cancer and viral diseases. A combined antiviral and antitumor effect of interferon is assumed to occur after surgical resection of hepatocellular carcinoma (HCC). Thus, IFN has a significant beneficial effect after curative treatment of HCC in terms of both survival and tumor recurrence [441]. Early preclinical studies demonstrated the importance of host immune mechanisms in the generation of long-lasting antitumor responses after type-I IFN treatment. More recent studies have revealed new immunomodulatory effects of IFN-α, including activities on T cells and dendritic cells. Overall, therapeutic strategies based on IFN-α include the use of these cytokines *in vivo* as immune adjuvants of cancer vaccines or their use *ex vivo* to generate DC-based vaccines and the combination of certain chemotherapy regimens with IFN-α [442-444]. Interferon-γ (IFN-γ) is a cytokine that acts on cell-surface receptors, activating transcription of genes that increase tumor immunogenicity, disrupt proliferative mechanisms and inhibit tumor angiogenesis. Current investigations of IFN-γ suggest that the cytokine has the potential to be used clinically in the treatment of brain tumors and as an adjuvant to other immunotherapeutic modalities [445]. The discovery of the interferon-λ (IFN-λ) family has considerably contributed the understanding of the role interferons play in viral infections and in cancer. The IFN-λ proteins, also termed interleukin-28 and -29, belong to the new type-III interferons. Type-III interferons are structurally similar to type-II IFN (IFN-γ) but functionally they are identical to

type-I IFN (IFN-α/β). The IFN-λ, have similar signaling pathways as IFN-α/β and they inhibit proliferation of tumor cells through cell cycle arrest or apoptosis. However, in contrast to type-I or -II IFNs, the response to type-III interferons is highly cell-type specific. Only epithelial cells and some immune cells respond to IFN-λ. This particular pattern of response is controlled by the differential expression of the IFN-λ receptor. Recently, the potent antitumor effects of IFN-λ were demonstrated, opening new opportunities for IFN therapy [446, 447].

Gene Therapeutics

Although the significance of MHC class I-restricted cytotoxic T lymphocytes (CTLs) as effectors of anti-tumor immunity has widely been demonstrated, most human tumors lack MHC-I expression or are inadequately differentiated and poorly immunogenic, a culprit that limits successful T-cell based tumor-specific immunotherapy. To overcome these disadvantages, the genetic modulation of T-lymphocytes using T cell receptor (TCR) transfer with tumor-specific TCR genes is an attractive strategy to generate anti-tumor responses, especially in large solid tumors. In this approach, the genes encoding a TCR specific for a defined antigen can be isolated from a T-cell clone and transduced to stimulated normal peripheral T-lymphocytes. This approach enables the redirection of the adaptive immune response against antigens of choice [448, 449]. A first demonstration of the fesability of this method was given by Morgan and coworkers who demonstrated that it is possible to transduce normal autologous PBLs from metastatic melanoma patients with a MART1-specific TCR and generate large numbers of MART1-specific cells to be infused back to the patients [450]. However, several factors may hold back the clinical benefit of this approach, such as the type of cells to modulate the vector configuration or the safety of the procedure.

The novel technique of RNA interference (RNAi), including small interfering RNA (siRNA), short hairpin RNA (shRNA) and microRNA (miRNA), mediate RNAi effects through the RNA inducible silencing complex RISC and represent attractive systems to be utilized as therapeutic tools [451]. Synthetic RNAs are nowadays widely used as tools for target validation and gene knock-down or knock-in. Presently, there is considerable interest for therapeutic applications of RNAi, particularly in areas of infectious disease and cancer. Preclinical data

demonstrate the efficacy of RNAi, for example knock-down of gene messages that are essential for tumor cell growth, metastasis, angiogenesis and chemoresistance, leading to anti-tumor effects. All types of RNA used for RNAi possess pharmacokinetic properties similar to single-stranded antisense oligonucleotides, but they are generally more robust than the latter [452, 453]. Despite all the potential of RNAi as a novel class of therapeutics, limited cellular uptake, low biological stability and unfavorable pharmacokinetic profiles are hampering their successful application in the clinic. Therefore, the translation of RNAi to the clinical setting is crucially dependent on the development of suitable delivery systems that improve their pharmacokinetic and biodistribution properties. Thus, delivery strategies for RNAi become the main hurdle that must be resolved prior to the full-scale clinical development of siRNA therapeutics [454-458]. As some examples, oncolytic adenoviral delivery of siRNA offers the potential benefits of restricted and renewable siRNA expression within the tumor microenvironment with an additive antitumor effect through viral oncolysis and siRNA-mediated oncogene silencing [459, 460]. Significant advances have been achieved with sterically stabilized lipid-based nanocarriers such as the stabilized nucleic acid lipid particles (SNALP). However, stabilization of nanocarriers with poly(ethylene glycol) (PEG) has not solved all problems associated with delivery of RNAi molecules. PEG modification weakens the internalization of the RNA molecules into the target cell and its subsequent escape from the endocytic pathway which reduces biological activity. To overcome such limitations novel exchangeable PEG-derivatized lipids can be used. After systemic administration, these lipids can be released from the nanoparticle surface. Additionally, the design and synthesis of cationic lipids that are more fusogenic and the use of internalizing targeting ligands have contributed to the emergence of novel lipid-based nanoparticles with remarkable transfection efficiency [461]. Finally, a nanoparticle formulation consisting of liposome-protamine-hyaluronic acid nanoparticles (LPH-NP) for systemic delivery of siRNA to tumors has been developed in a self-assembling process. The LPH-NP was further modified by PEG or PEG-anisamide lipids. Anisamide is a targeting ligand for the sigma receptor over-expressed in B16F10 melanoma cells. The targeted LPH-NP silenced 80% of luciferase activity in metastatic B16F10 lung tumors after a single i.v. injection and also showed very little immunotoxicity [462].

TME Mediated Drug Resistance

Resistance against antitumor drugs and therapeutic radiation represents a tremendous challenge for most cancer therapies. It has been demonstrated by various experimental approaches that the mesenchymal TME provides a protective environment that obstructs drug or radiation access to the tumor cells or creates a permissive environment that supports for example the existence of cancer stem cell (CSC) niches where tumor cells overcome treatment- and cancer-induced stresses [463-471]. Resistance of tumors to anticancer drugs is mostly attributed to gene mutations, amplification of the multidrug resistance genes, epigenetic changes that influence drug uptake and metabolism, or export of drugs from cells [472-474]. An important advance in the understanding of tumor multidrug resistance (MDR) came with the identification of the P-glycoproteins (ABC transporter family) and other related transporters that are expressed in cancer cells and orchestrate the efflux of drugs from cells [475-477]. Tumor cells can also undergo physiologic changes in response to extracellular acidosis, a consequence of high glycolytic flux and poor vascular perfusion, both of which contribute to drug resistance including reduced apoptotic potential, genetic alterations, and elevated P-glycoprotein levels. A low extracellular pH creates a physiological drug barrier described by an "ion trapping" phenomenon [478, 479]. In addition, unfavorable pharmacokinetics and -dynamics and the limited ability of cancer drugs to diffuse deeply into hypoxic tumor tissue and to accumulate in tumor cells at lethal concentrations contributes to the unsatisfactory efficacy of cancer therapy [480, 481].

Regarding the contribution of stromal cells in the induction of drug resistance, increased infiltration of macrophages and high cathepsin protease levels in TAM were found in tumors following chemotherapy with paclitaxel, etoposide and doxorubicin, suggesting that cathepsin-expressing macrophages protected tumor cells against drug-induced tumor cell death [482]. It was also reported that TAM and their expression of milk-fat globule-epidermal growth factor-VIII (MFG-E8) play a role in the regulation of CSC. MFG-E8 activates Stat3 and Sonic Hedgehog pathways in CSC and further amplifies their anticancer drug resistance in cooperation with IL-6 [483]. The contribution of cancer associated fibroblasts (CAF) in induction of drug resistance was recently demonstrated in a co-culture

study of estrogen receptor positive MCF7 breast cancer cells with fibroblasts showing that tamoxifen resistance was induced by CAF. The fibroblasts also protected MCF7 cells against apoptosis induced by other anticancer agents, such as doxorubicin and the PARP-1 inhibitor ABT-888 [484].

Work in several different cancers has suggested that the CSC population serves as a source of chemotherapy and radiation-therapy resistance within tumors. Several resistance mechanisms have been proposed, including amplified checkpoint activation and DNA damage repair as well as increased Wnt/β-catenin and Notch signaling. Targeted therapies against the DNA damage checkpoint or stem-cell maintenance pathways may sensitize CSC to radiation or other therapies. CSC may also play a role in the induction of angiogenesis as well as in the mechanisms of resistance towards anti-angiogenic agents [485]. The dynamics of cancer cell death in response to therapy was recently investigated by intravital microscopy of chemotherapy-treated mouse tumors allowing a dynamic analysis of drug distribution, cell death and tumor-stroma interactions. Thereby, associations between vascular leakage and response to doxorubicin, including improved response in MMP-9 knockout mice that had increased vascular leakage were observed. Furthermore, CCR2-dependent infiltration of myeloid cells after treatment and better response of Ccr2 null host mice to doxorubicin and cisplatin treatment was demonstrated [486].

In respect to anti-angiogenic therapies, inhibitors targeting the VEGF signaling pathways have demonstrated, in both preclinical and clinical settings, that the benefits are at best transitory and often followed by re-establishment of tumor growth and progression. Several findings support the notion that two modes of unconventional resistance underlie such results; either the mode of evasive resistance, which is an adaptation to circumvent the specific angiogenic blockade, or an intrinsic or pre-existing indifference towards anti-angiogenic drugs [487-490]. Emerging evidence indicates that anti-angiogenic agents may increase intratumor hypoxia by promoting vessel pruning and inhibiting neo-angiogenesis. Indeed, several studies have highlighted the possibility that VEGF and VEGF-receptor inhibition can promote an invasive metastatic switch, in part by creating an increasingly hypoxic tumor microenvironment. As a potential remedy, a number of therapeutic approaches have been investigated that target the hypoxic tumor compartment to improve the clinical outcome of anti-angiogenic therapy [491-493].

Novel approaches to control drug resistance include functional genomics and proteomics [494,495]. RNA interference based screening provides a valuable opportunity for the examination of intrinsic and acquired resistance mechanisms. The availability of short interfering RNA libraries targeting genes allows performing large-scale screens to identify molecules that are involved in multidrug resistance pathways [496]. The emerging role of microRNAs as key gene expression regulators is also being explored in drug resistance research [497]. Finally, immunotherapy could represent an important adjuvant to treat MDR, as resistance to immunotherapy generally is unrelated to the classical mechanisms of resistance to cytotoxic agents. Immunotherapy to combat MDR could consist of direct immune attack against MDR positive cells, using MDR as an immune target to deliver cytotoxic drugs, taking advantage of other immune properties of MDR positive cells or application of immunotoxins expressed under MDR control [498, 499]. Regarding therapeutic approaches against drug resistance, nanodrug carriers, in particular liposomes, are widely explored [500-502]. Nanocarrier strategies for the reversal of resistance involve the alteration of drug efflux pumps and other resistance mechanisms. The methodologies involved include specific targeting of drugs and nucleotide therapeutics, improvement of cellular uptake and bioavailability of drugs with poor physicochemical characteristics. Multifunctional nanoparticulate systems consisting of a targeting moiety, encapsulated cytotoxic drugs and an element responsive to the TME to release the encapsulated therapeutics hold promise toward ways to improve cancer treatment [503-505].

CONCLUSION

The cancer cell centric view of tumor progression largely ignored for a long time the fact that complex interactions between cancer cells and the cellular and molecular components of the tumor microenvironment tightly regulate and orchestrate tumor growth, metastatic dissemination and in many instances also the outcome of cancer therapies. Despite of continuous efforts, for many years cancer research largely focused on cancer-cell driven carcinogenesis and on understanding the mutations causing neoplastic cell transformations. But to provide new therapeutic strategies targeted at the immune components of the TME, it is critical to understand how these cells are altered during tumor progression and how they reciprocally influence tumor initiation, progression and

metastasis. The three mainstays of cancer therapy, surgical removal of tumor tissue, chemotherapy and radiotherapy will be complemented in the future by a fourth pillar, namely tumor immunotherapy and novel treatments aimed at the cellular and molecular components of the tumor microenvironment. Such novel strategies are urgently needed to complement the classical treatment modalities with more effective and patient tailored therapeutic approaches.

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CONFLICT OF INTEREST

The authors confirm that this chapter contents have no conflict of interest.

DISCLOSURE

Declared none.

ABBREVIATIONS

ADEPT = Antibody-directed enzyme prodrug therapy

Arg = Arginase

bFGF = Basic fibroblast growth factor

BP = Bisphosphonate

CAF = Cancer-associated fibroblast

CCL = Chemokine (C–C motif) ligand

CCR = Chemokine (C–C motif) receptor

CSC = Cancer stem cell

CSF-1 = Colony-stimulating factor 1

CTL = Cytotoxic T-lymphocyte

CXCL = Chemokine (C–X–C motif) ligand

DC = Dendritic cell

EC = Endothelial cell

ECM = Extracellular matrix

EGF = Epidermal growth factor

EGF = Epidermal growth factor

EMT = Epithelial-mesenchymal transition

FGF = Fibroblast growth factor

GDEPT = Gene-directed enzyme prodrug therapy

GM-CSF = Granulocyte–macrophage colony-stimulating factor

Gr1 = Granulocyte differentiation antigen

HGF = Hepatocyte growth factor

HGF = Hepatocyte growth factor

HIF-1 = Hypoxia inducible factor 1

IFN-γ = Interferon γ

IL = Interleukin

iNOS = Inducible nitric oxide synthase

LAK = Lymphokine activated killer cell

LPS = Lipopolysaccharide

M1 = Classically activated macrophages

M2 = Alternatively activated macrophages

MC = Mast cell

MDSC = Myeloid derived suppressor cell

MHC = Major histocompatibility complex

miRNA = MicroRNA

MMP = Matrix metalloproteinase

MR = Mannose receptor

MSC = Mesenchymal stem cell

N1 = Classically activated neutrophil

N2 = Alternatively activated neutrophil

N-BP = Nitrogen-containing bisphosphonate

NF-κB = Nuclear factor kappaB

NO = Nitric oxide

non-N-BP = Non-nitrogen containing bisphosphonate

PDGF = Platelet-derived growth factor

PDGF = Platelet derived growth factor

PMT = Prodrug monotherapy

RAIT = Radioimmunotherapy

RNAi = RNA interference

ROI = Reactive oxygen intermediate

ROS = Reactive oxygen species

shRNA = Short hairpin RNA

siRNA = Small interfering RNA

STAT = Signal transducer and activator of transcription

TADC = Tumor-associated dendritic cell

TAM = Tumor-associated macrophage

TAN = Tumor-associated neutrophil

TGF-α = Transforming growth factor α

TGF-β = Transforming growth factor-β

T_h = Helper T-cell

TIL = Tumor infiltrating leucocyte

TME = Tumor microenvironment

TNF-α = Tumor necrosis factor-α

TNF-α = Tumor necrosis factor α

T_{reg} = Regulatory T-cell

uPA = Urokinase-type plasminogen activator

VEGF = Vascular endothelial growth factor

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